This is a metadata sheet with extra info on sampling and processing of the FlowCAM dataset			
Please note that over the years several aspects of the sampling and processing have been fine-tuned and optimized.			

2017

Sampling	started May 2017		
	replicates	1 sample/station	
	volume_filtered	50L or 70L	depending on what was possible (suspended matter clogging the net, time constraints, ...)
	sampling_method	buckets	
	sampling_depth	surface water	
	Apstein_net	55µm	1.2 m long and 0.50 m diameter
	fixative	neutral Lugol iodine's solution	https://www.chemlab-analytical.be/#/en-gb/prod/1392739
	concentration_fixative	2-5%	Cognac color
	storage	4°C, dark	
Processing	objective	4x	
	flowcell	300FC	
	collimator	no	
	syringe_pump	5 ml	
	pipette_tip	5 ml	
	filter_mesh_size	300 µm	Pre-filter before sample is processed to avoid flowcell clogging.
	flow_rate	1,7 ml/min	
	autoimage_rate	20 fps	
	sampling_volume	5ml	
	stop_criterium	1500 particles or 5ml	
	pseudoreplicates	3 runs/sample	Between each run and each sample, the system is flushed with 3x 5ml distilled water and 3x ethanol 70% (alternating, ending with distilled water).
	focus	manual	
	camera	Sony XCD SC90	
	color_grade_camera	grey scale	
	flowcam_model	Flow Cytometer and Microscope (FlowCAM®) VS-4 (Fluid Imaging Technologies, Yarmouth, Maine, U.S.A.)	
	processing_time	5 min/run	30 min/sample total
	imaging_mode	Autoimage mode	
	PPUI	1.10-1.20	
	Basic_aquisition_filter	50-300 ESD	
	software	VisualSpreadsheet® Version 4.2.52.	
Labeling	VisualSpreadsheet		

2018

Sampling			
	replicates	1 sample/station	
	volume_filtered	50L or 70L	depending on what was possible (suspended matter clogging the net, time constraints, ...)
	sampling_method	buckets	
	sampling_depth	surface water	
	Apstein_net	55µm	1.2 m long and 0.50 m diameter
	fixative	neutral Lugol iodine's solution	https://www.chemlab-analytical.be/#/en-gb/prod/1392739
	concentration_fixative	2-5%	Cognac color
	storage	4°C dark	
Processing			
	objective	4x	
	flowcell	300FC	
	collimator	no	
	syringe_pump	5 ml	
	pipette_tip	5 ml	
	filter_mesh_size	300 µm	Pre-filter before sample is processed to avoid flowcell clogging.
	flow_rate	1,7 ml/min	
	autoimage_rate	20 fps	
	sampling_volume	8 ml	
	stop_criterium	1500 images or 8 ml	

	pseudoreplicates	3 runs/sampled	Between each run and each sample, the system is flushed with 3x 5ml distilled water and 3x ethanol 70% (alternating, ending with distilled water).
	focus	manual	
	camera	Sony XCD SC90	
	color_grade_camera	grey scale	
	flowcam_model	Flow Cytometer and Microscope (FlowCAM®) VS-4 (Fluid Imaging Technologies, Yarmouth, Maine, U.S.A.)	
	processing_time	9 min/run	30 min/sample total
	imaging_mode	Autoimage mode	
	PPUI	1.10-1.20	
	Basic_aquisition_filter	50-300 ESD	
	software	VisualSpreadsheet® Version 4.2.52.	
Labeling	VisualSpreadsheet		

2019-2020

Sampling			
	replicates	1 sample/station	
	volume_filtered	50L	
	sampling_method	buckets	
	sampling_depth	surface water	
	Apstein_net	55µm	1.2 m long and 0.50 m diameter
	fixative	neutral Lugol iodine's solution	https://www.chemlab-analytical.be/#/en-gb/prod/1392739
	concentration_fixative	2-5%	Cognac color
	storage	4°C dark	
Processing			
	objective	4x	
	flowcell	300FC	
	collimator	no	
	syringe_pump	5 ml	
	pipette_tip	5 ml	
	filter_mesh_size	300 µm	Pre-filter before sample is processed to avoid flowcell clogging.
	flow_rate	1,7 ml/min	
	autoimage_rate	20 fps	
	sampling_volume	8ml	
	stop_criterium	1500 images or 8ml	
	pseudoreplicates	3 runs/sample	Between each run and each sample, the system is flushed with 3x 5ml distilled water and 3x ethanol 70% (alternating, ending with distilled water).
	focus	manual	
	camera	Sony XCD SC90	
	color_grade_camera	grey scale	
	flowcam_model	Flow Cytometer and Microscope (FlowCAM®) VS-4 (Fluid Imaging Technologies, Yarmouth, Maine, U.S.A.)	
	processing_time	9 min/run	30 min/sample total
	imaging_mode	Autoimage mode	
	PPUI	1.10-1.20	
	Basic_aquisition_filter	50-300 ESD	
	software	VisualSpreadsheet® Version 4.2.52.	
Labeling	CNN + manual validation		